Biomimetic Membrane Transport: Interesting Ionophore Functions of Naturally Occurring Polyether Antibiotics toward Unusual Metal Cations and Amino Acid Ester Salts

Hiroshi Tsukube,* Kentaro Takagi, Tatsuo Higashiyama, Tadashi Iwachido, and Naomi Hayama

Department of Chemistry, College of Liberal Arts & Science, Okayama University, Okayama 700, Japan

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In an effort to develop new functionalities of naturally occurring ionophores, we designed a new "biomimetic membrane transport" system mediated by monensin, lasalocid, and salinomycin. Under nonbiological conditions, these ionophores specifically transported unnatural guest species such as amino acid ester salts and heavy and transition metal cations. Liquid–liquid extraction and ^{13}C-NMR binding experiments clearly demonstrated that these biological ionophores accommodated various guest cations in their pseudocavities and that the overall transport rate was significantly determined by a combination of rates of guest binding and releasing processes. Several biomimetic applications can be envisaged using these ionophores in the border regions between synthetic and biological supramolecular chemistry.

Introduction

Polyether antibiotics such as monensin (**1**) and lasalocid (**3**) which are isolated from *Streptomyces cinnamonensis* and *lasaliensis* form a well-defined class of naturally occurring carrier molecules for alkali and alkaline earth metal cations. They are called ionophores and are characterized by an acyclic polyether chain terminated on one side by a carboxylic function and on the other by one or two hydroxyl groups. Crystallographic studies of their metal complexes showed a pseudocyclic conformation in which both ends of the polyether chain are linked by intramolecular head to tail hydrogen bonds. The guest cations are located at the center of the pseudocavity and coordinated by several oxygen atoms. In this respect their complex structures are closely similar to those of crown ethers and other synthetic macrocycles.[1-3]

The stability constants of metal complexes with various polyether antibiotics have been measured in organic media using many different techniques.[4] Interestingly, the results revealed that some naturally occurring ionophores form very stable complexes with cationic species other than their biological guests. Typically, monensin (**1**) shows high binding abilities for K^+, Rb^+, and Ag^+ ions in ethanol solution, though it specifically transports a Na^+ ion across a biomembrane.[5] These unexpected but characteristic features of polyether antibiotics strongly suggest that they may have ligand arrangements flexible enough to adjust to some unusual guest cations and potential as a new class of carriers for nonbiological guest cations. Westley et al. demonstrated that lasalocid formed crystalline complexes with several asymmetric amine salts.[6] We[7] and Lindoy et al.[8] successfully employed polyether-type antibiotics as the carriers for liquid membrane transport. More recently, we succeeded in deriving chiral receptors from monensin and related biological ionophores which offered enantiomer selective complexations with amino acid ester salts.[9-12] Suzuki et al. also modified some polyether-type antibiotics to give sensing molecules for ion-selective electrodes.[13]

In this paper, we formulate a unique biomimetic transport system using naturally occurring monensin (**1**), lasalocid (**3**), and salinomycin (**4**) (see Chart 1).[7] Under nonbiological conditions, these were demonstrated to accommodate unusual guest cations in their pseudocavities. Although ambitious approaches are currently being made toward the biological functions of naturally occurring ionophores, we present a unique successful example of an artificial liquid membrane system in which naturally occurring ionophores show excellent binding and transport abilities for unnatural guest cations. The results obtained here, therefore, promise not only greater understanding of biomembrane transport but also new ideas in the design of practical applications of biological ionophores.

Results and Discussion

Biomimetic Transport System. Figure 1 illustrates the new biomimetic transport system mediated by naturally occurring polyether antibiotics, comparing it with a biological transport system. In biological transport, the polyether antibiotic mediates an antiport of a guest metal cation as follows.[14] At the aq. 1 (basic)/membrane interface, a carboxylic acid-type polyether forms an electrically neutral complex with a guest cation which is accommodated in a characteristic pseudocavity. The resulting complex taken up in the membrane moves to the other side. At the membrane/aq. 2 (acidic) interface, the guest cation is released after neutralization of the carboxylic acid polyether. Thus, a pH gradient across the membrane promotes effective cation transport. Our developed biomimetic transport, on the other hand, involves the cation exchange of a pseudocyclic metal complex. A polyether complex with guest cation is formed at the aq. 1 (neutral)/

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(1) Hilgenfeld, R.; Saenger, W. *Topics in Current Chemistry*; Springer-Verlag: Berlin, 1981; Vol. 101, p 1.
(2) Painter, G. R.; Pressman, B. C. *Topics in Current Chemistry*; Springer-Verlag: Berlin, 1981; Vol. 101, p 83.
(3) Tsukube, H. *Cation-Binding by Macrocycles*; Marcel-Dekker: New York, 1990; p 497.
(4) Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J. *Chem. Rev.* **1985**, *85*, 271.
(5) Cox, B. G.; Trung, N.; Rzeszotarska, J.; Schneider, H. *J. Am. Chem. Soc.* **1984**, *106*, 5965.
(6) Westley, J. W.; Evans, R. H.; Blout, J. F. *J. Am. Chem. Soc.* **1977**, *99*, 6057.
(7) Preliminary communication of this article: Tsukube, H.; Takagi, K.; Higashiyama, T.; Iwachido, T.; Hayama, N. *J. Chem. Soc., Chem. Commun.* **1986**, 448.
(8) They applied the lasalocid ionophore to the liquid membrane transport of metal ammine and amine complexes: Lindoy, L. F.; Walker, G. W.; Everett, G. W. *J. Am. Chem. Soc.* **1990**, *112*, 3659. Chia, P. S. K.; Lindoy, L. F.; Walker, G. W.; Everett, G. W. *J. Am. Chem. Soc.* **1991**, *113*, 2533.
(9) Maruyama, K.; Sohmiya, H.; Tsukube, H. *J. Chem. Soc., Chem. Commun.* **1989**, 864.
(10) Tsukube, H.; Sohmiya, H. *Tetrahedron Lett.* **1990**, *31*, 7027.
(11) Tsukube, H.; Sohmiya, H. *J. Org. Chem.* **1991**, *56*, 876.
(12) Tsukube, H.; Sohmiya, H. *Supramol. Chem.* **1993**, *1*, 297.
(13) Tohda, K.; Suzuki, K.; Kosuge, N.; Watanabe, K.; Nagashima, H.; Inoue, H.; Shirai, T. *Anal. Chem.* **1990**, *62*, 936.
(14) Choy, E.; Evans, D. F.; Cussler, E. L. *J. Am. Chem. Soc.* **1974**, *96*, 7085.

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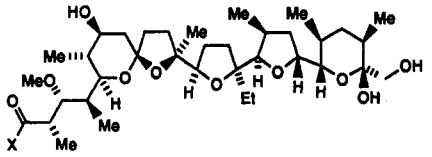
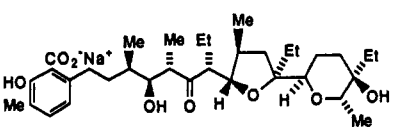
Chart 1. Molecular Structures of Examined Naturally Occurring Ionophores **1**, **3–5**, and Monensin Ester **2**



1: X= O^-Na^+

2: X= OCH_3



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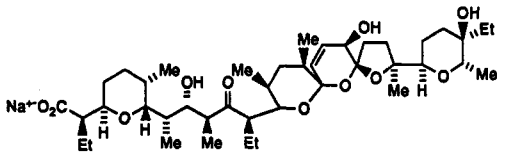

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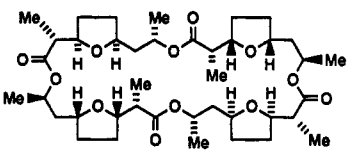

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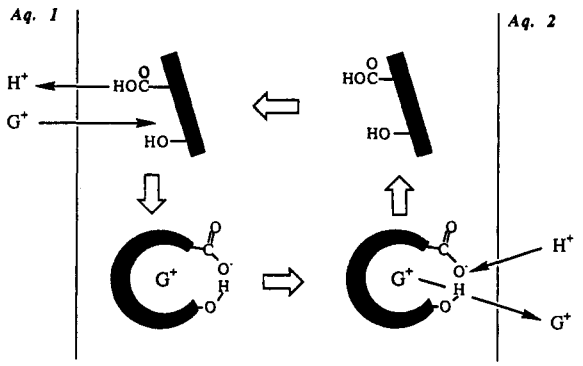
"BIOLOGICAL TRANSPORT"



"BIOMIMETIC TRANSPORT"

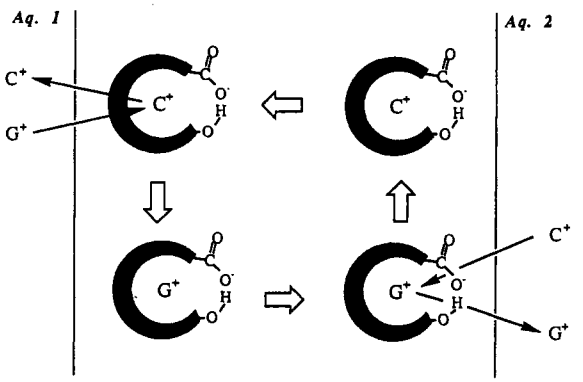


Figure 1. Representation of cation transport across a liquid membrane: biological transport (upper) and biomimetic transport (lower). H^+, proton; G^+, guest cation; C^+, countertransported cation.

membrane interface and then diffuses across the membrane. At the membrane/aq. 2 (neutral) interface, a cation-exchange reaction with countertransported cation releases guest cation. As a result, guest and countertransported cations are transported through the membrane in opposite directions. Since the driving force of this biomimetic transport is the concentration gradient of the countertransported cation, pH gradient is not required.

Transport Studies. As carriers for the new biomimetic membrane transport, we employed naturally occurring monensin (**1**), lasalocid (**3**), and salinomycin (**4**), which are typical carboxylic acid-type polyether antibiotics and mediate biological transport of Na^+, Cs^+, and K^+ ions, respectively. Monensin methyl ester (**2**) and macrocyclic nonactin (**5**) were also examined for comparison, because they are neutral but have similar polyether sequences. Typical results of biomimetic transport, obtained using a $CHCl_3$ liquid membrane, are summarized in Table 1.

Table 1. Biomimetic Transport Mediated by Naturally Occurring Ionophores[a]

		transport rate × 10^6 (mol/h)				
guest cation	countertransported cation	**1**	**2**	**3**	**4**	**5**
TrpOEtH$^+$ [b]	none	0	0	0	0	0
	Li^+	0.22	0	0.18	0.31	0
	Na^+	2.82	0	0.23	0.67	0
	K^+	0.85	0	0.54	2.13	0
	NH_4^+	0.22	0	0.76	0.90	0
PheOEtH$^+$ [c]	Na^+	1.23	0			
	K^+				1.10	0
	NH_4^+			0.28		
TyrOEtH$^+$ [d]	Na^+	2.73	0			
	K^+				6.41	0
	NH_4^+			1.57		
Ag^+	Na^+	1.77	0.90			
	K^+				2.13	0
	NH_4^+			4.84		
Pb^{2+}	Na^+	2.00	0			
	K^+				2.80	0
	NH_4^+			0.34		
Cu^{2+}	Na^+	0	0			
	K^+				0*	0
	NH_4^+			1.53		
Ni^{2+}	Na^+	0	0			
	K^+				0	0
	NH_4^+			3.33		
Co^{2+}	Na^+	0.09	0			
	K^+				0.02	0
	NH_4^+			1.57		
Zn^{2+}	Na^+	0.30	0			
	K^+				0.33	0
	NH_4^+			1.67		

[a] Transport conditions: aq. 1, amine hydrochloride or metal perchlorate, 0.5 mmol/H_2O, 5 mL; membrane, ionophore 0.0327 mmol/$CHCl_3$, 12 mL; aq. 2, countertransported cation chloride, perchlorate or nitrate, 2.50 mmol/H_2O, 5 mL. Indicated values were calculated from the initial rates of appearance of guest cations in the aq. 2 phase. Reproducibilities were 15% or better. A blank table entry indicates not attempted. An asterisk indicates turbidity appeared. [b] Tryptophan ethyl ester (protonated form). [c] Phenylalanine ethyl ester (protonated form). [d] Tyrosine ethyl ester (protonated form).

Monensin (**1**) and salinomycin (**4**) effectively transported several amino acid ester salts and Ag^+ and Pb^{2+} cations under the biomimetic transport conditions employed, while Cu^{2+}, Ni^{2+}, and other transition metal cations were hardly transported at all (Table 1). Their transport profiles were apparently controlled by the "cation–cavity size concept" established in crown ether

Table 2. Extraction Profiles of Naturally Occurring Ionophores[a]

ionophore	additive in aq phase	guest cation/ionophore (%) in $CHCl_3$						
		$TrpOEtH^+$	Ag^+	Pb^{2+}	Cu^{2+}	Ni^{2+}	Co^{2+}	Zn^{2+}
1	none	81	83	40	34*	14*	10*	20
	$NaClO_4$ (0.05 M)	45	60	24	16	2	2	2
3	none	94	85	48	46	48	48	48
	NH_4ClO_4 (0.05 M)	92	60	42	24	6	6	22
4	none	94	86	40	28*	22	18	38
	KNO_3 (0.05 M)	75	72	38	32*	10	6	16
5	none	5	4	0	4	2	0	0

[a] Conditions: aqueous phase, TrpOEt·HCl or $MClO_4$, 0.01 mmol, additive, 0.10 mmol/H_2O, 2 mL; $CHCl_3$ phase, ionophore, 0.01 mmol/$CHCl_3$, 1 mL. An asterisk indicates considerable amounts of precipitate appeared.

chemistry.[15] The CPK molecular model of monensin (**1**) strongly suggests that its pseudocavity consists of an approximately 17-membered ring with six oxygen donor atoms and compares well in size with the effectively transported organic ammonium, Ag^+, and Pb^{2+} cations. Cu^{2+}, Ni^{2+}, and other transition metal cations, however, seem too small to interact effectively with polyether oxygen atoms and therefore are rarely transported by monensin (**1**). Salinomycin (**4**) is also thought to have a pseudocavity to accommodate guest cations of similar sizes. This effectively transports $TrpOEtH^+$, Ag^+, and Pb^{2+} ions across an artificial liquid membrane, while it specifically mediates biological transport of K^+ ion.

Lasalocid (**3**) exhibited apparently cation transport selectivities different from those observed with monensin (**1**) and salinomycin (**4**). Higher transport rates were observed with Ag^+, Ni^{2+}, Zn^{2+}, and Co^{2+} cations than with amino acid derivatives (Table 1). Since the salicylic acid moiety of lasalocid is known to act as a potential binding site for heavy and transition metal cations, its unique coordination ability provided characteristic transport phenomena. Neutral monensin methyl ester (**2**) and macrocyclic nonactin (**5**) were also examined as carriers for biomimetic transport. They have been reported to mediate symport of several cationic species effectively[16,17] but seldom transported any guest cations under the antiport conditions employed. Therefore, the molecular structure of the employed ionophore is the most essential factor in determining efficiency and selectivity of this biomimetic transport system. Acyclic polyether chains having carboxylic acid terminal groups are clearly suitable as the carrier skeletons.

Table 1 also indicates that the nature of countertransported cation influences overall transport rate: the Na^+ cation was more effective for monensin-mediated biomimetic transport than Li^+, K^+, and NH_4^+ ions. Since monensin (**1**) binds Na^+ cation more tightly, it could effectively accelerate the cation-exchange process at the membrane/aq. 2 interface (Figure 1). We confirmed that the transported amount of guest cation was almost equal to that of countertransported cation and that pH values of the aq. 1 and aq. 2 phases slightly changed during the experiments.[18] These observations strongly suggest that the present biomimetic transport proceeds via an antiport mechanism as shown in Figure 1.

Extraction Studies. To examine the cation binding properties of naturally occurring ionophores, we performed liquid–liquid extraction experiments using $TrpOEtH^+$, Ag^+, Pb^{2+}, Cu^{2+}, Co^{2+}, and Zn^{2+} cations. The extraction abilities of these cations were estimated on the basis of the partition of the cation between chloroform and aqueous solutions, and typical results are summarized in Table 2.

The polyether antibiotics **1**, **3**, and **4** having carboxylate anions at the terminal groups exhibited generally excellent extraction abilities for various guest cations, though neutral macrocyclic nonactin (**5**) extracted them only poorly. Electrostatic interactions between the anion-charged polyether and guest cation were significantly involved in the extraction process. For example, monensin (**1**) showed very high extractabilities for the $TrpOEtH^+$, Ag^+, and Pb^{2+} cations, supporting a 1:1 complexation (guest cation:ionophore) for $TrpOEtH^+$ and Ag^+ ions and a 1:2 complexation (guest cation:ionophore) for Pb^{2+} ion.[19] Interestingly, the extracted amounts of several guest cations were definitely reduced in the presence of Na^+ cation. Since these cation extraction trends were parallel to the transport profiles as mentioned above, the $TrpOEtH^+$, Ag^+, and Pb^{2+} ions were believed to be smoothly bound and easily released at both membrane interfaces. Lasalocid (**3**) showed somewhat different extraction behaviors. **3** offered higher extraction abilities for all the examined cations than monensin (**1**) but only weakly transported the $TrpOEtH^+$ and Pb^{2+} cations. Tables 1 and 2 indicate that the guest cations which complexed moderately with the ionophore were rapidly transported. A similar correlation between transport rate and extraction ability was observed in the salinomycin-mediated system. $TrpOEtH^+$, Ag^+, and Pb^{2+} ions were suitable guest cations for effective transport in this case.

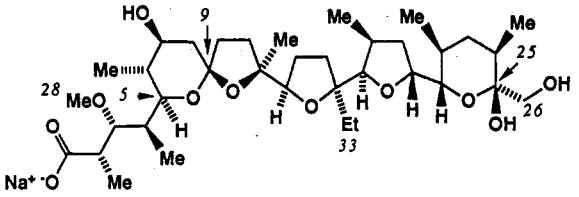
^{13}C-NMR Binding Studies. The complexation behaviors of the naturally occurring ionophores with several guest cations were investigated by means of ^{13}C-NMR spectroscopy. We shook an aqueous solution of guest salt with a $CDCl_3$ solution containing the sodium salt of monensin (**1**) or lasalocid (**3**). Tables 3 and 4 summarize the ^{13}C-NMR chemical shifts of the selected carbon signals of monensin (**1**) and lasalocid (**3**) after extraction experiments. Several spectral changes were observed upon cation exchange. The signal assignments were based on the reported assignments for monensin (**1**)[20] and lasalocid (**3**).[21] Numberings of the carbons are indicated in the structural formulas described in Tables 3 and 4.

Tables 3 and 4 indicate that the $TrpOEtH^+$ cation induced significant changes in the signals for several carbons around their pseudocavities. Since the extraction experiment (Table 2) revealed that the ionophores **1** and **3** strongly bound the $TrpOEtH^+$ cation, these spectroscopic results confirmed that they could adjust their conformations to accommodate the $TrpOEtH^+$ cation in their pseudocavities.[22]

Ag^+ and Zn^{2+} ions also induced characteristic spectral changes of these ionophores. In particular, aromatic carbon signals of lasalocid (**3**) were greatly shifted upon complexation. This

(15) Tsukube, H. *Crown Ethers and Analogous Compounds*; Elsevier: Amsterdam, 1992; p 100.
(16) Tsukube, H.; Sohmiya, H. *Inorg. Chim. Acta* **1989**, *166*, 27.
(17) Tsukube, H.; Takagi, K.; Higashiyama, T.; Iwachido, T.; Hayama, N. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2021.
(18) The transported amount of H^+ from aq. 2 to aq. 1 was negligibly small as compared with that of guest cation. The nature of the counteranion rarely influenced the overall transport rate.
(19) The counteranion, ClO_4^-, was confirmed to be hardly extracted from the aqueous phase into $CHCl_3$, supporting a 1:2 stoichiometry. Some polyether ionophores exist as both monomers and dimers in complexes: Johnson, S. M.; Herrin, J.; Lui, S. J.; Paul, J. C. *J. Chem. Soc., Chem. Commun.* **1970**, 72. Schmidt, P. G.; Wang, A. H.-J.; Paul, I. C. *J. Am. Chem. Soc.* **1974**, *96*, 6189. Lyazghi, R.; Pointud, Y.; Dauphin, G.; Juillard, J. *J. Chem. Soc., Perkin 2* **1993**, 1681.
(20) Ajaz, A. A.; Robinson, A.; Turner, D. L. *J. Chem. Soc., Perkin 1* **1987**, 27.
(21) Lyazghi, R.; Cuer, A.; Dauphin, G.; Juillard, J. *J. Chem. Soc., Perkin 2* **1992**, 35.
(22) The chemical shifts for the carbon signals of $TrpOEtH^+$ bound with monensin (**1**) were different from those with lasalocid (**3**). In particular, indole ring carbon signals sensitively shifted.

Table 3. ^{13}C-NMR Chemical Shifts of Monensin (1) after Extraction[a]

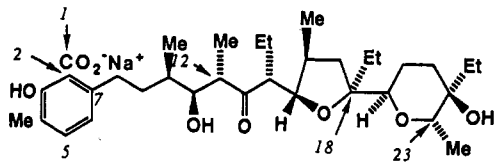


1

guest cation	chemical shift (ppm)						
	C_1	C_9	C_{25}	C_5	C_{26}	C_{28}	C_{33}
Na^+	181.4	107.1	98.5	68.5	65.0	57.9	8.2
$TrpOEtH^+$	177.3	108.0	98.5	67.9	67.2	58.1	8.6
Ag^+	181.7	108.2	97.8	68.5	65.1	58.0	8.4
Zn^{2+}	181.4	107.1	98.5	68.5	65.0	57.9	8.2

[a] Conditions: Monensin sodium salt, 0.03 mmol in $CDCl_3$, 1 mL; $NaClO_4$, TrpOEt·HCl, $AgClO_4$, or $Zn(ClO_4)_2$, 0.03 mmol in H_2O, 2 mL.

Table 4. ^{13}C-NMR Chemical Shifts of Lasalocid (3) after Extraction[a]



3

guest cation	chemical shift (ppm)						
	C_1	C_7	C_5	C_2	C_{18}	C_{23}	C_{12}
Na^+	176.3	143.5	131.7	119.8	87.2	76.8	55.8
$TrpOEtH^+$	175.6	144.4	132.1	120.0	87.6	76.8	56.4
Ag^+	176.1	144.0	132.2	119.5	86.6	77.1*	55.7
Zn^{2+}	177.0	144.3	133.4	120.8	86.2	76.8	55.5

[a] Conditions: Same as those indicated in Table 3. An asterisk indicates this signal was overlapped with that of the solvent ($CDCl_3$).

indicates that the salicylic acid moiety of lasalocid plays an important role in the binding of heavy and transition metal cations.[23] The carbon signals of monensin (**1**) modestly shifted in the presence of Ag^+ ion. Since monensin extracted this effectively, a slight conformational change should be required prior to exchange of Na^+ ion for Ag^+ ion. Thus, polyether-type ionophores were confirmed to have ligand arrangements flexible enough to accommodate several nonbiological guest cations.

These cation-binding and transport results clearly demonstrated that several naturally occurring polyether antibiotics exhibited interesting ionophore functions toward unnatural guest species and strongly suggest that other kinds of biological materials may offer further interesting functionalities in a variety of artificial processes.

Experimental Section

All the biological ionophores examined were purchased from Calbiochem Corp., Sigma Chemical Co., Wako Pure Chemical Industries, and Fluka AG. Special care is required in their usages and disposal. Solvents and other reagents were also commercially available and used without additional purification.

^{13}C-NMR spectra were recorded on a Varian UXR-500 and a Jeol 90A. The concentration of each metal cation in aqueous solution was determined by atomic absorption and flame spectroscopic methods (performed at Exlan Technical Center Co., Okayama, Japan). We also used a UV spectrometer (Shimadzu UV-365) to determine the concentrations of theTrpOEt salt and ion-selective electrodes for determination of ClO_4^- and Cl^- anions.

Transport Experiments. Transport experiments were performed at room temperature (ca. 16 °C) in a U-tube glass cell (2.0 cm, i.d.).[24] The ionophore (0.0372 mmol), dissolved in chloroform (12 mL), was placed in the base of the tube, and two aqueous phases (aq. 1, guest cation chloride or perchlorate, 0.5 mmol/H_2O, 5 mL; aq. 2: countertransported cation chloride, nitrate, or perchlorate, 2.5 mmol/H_2O, 5 mL) were placed in the arms of the U-tube, floating on the chloroform membrane. The membrane phase was stirred constantly with a magnetic stirrer. The transport rates indicated in Table 1 were calculated from the initial rates of appearance of guest cation into the aq. 2 phase. The transported amount of countertransported cation into the aq. 1 phase was confirmed as being almost equal to that of the guest cation in aq. 2. Reproducibilities were confirmed as 15% or better.

Extraction Experiments. Extraction experiments were conducted by adding a chloroform (or $CDCl_3$) solution of ionophore to an aqueous solution of tryptophan ethyl ester hydrochloride or metal perchlorate both in the presence and absence of $NaClO_4$ and other additives. After the mixture had been stirred for 2 h, the aqueous phase was separated and the concentration of each guest cation in this phase was determined spectroscopically. Details of the extraction conditions are indicated as a footnote to Table 2, which summarizes the average values of two independent experiments. We obtained almost the same results after 3 h of stirring.

^{13}C-NMR Experiments. The chemical shifts indicated in Tables 3 and 4 were determined by using the central peak of the $CDCl_3$ carbon (δc 77.10 ppm) as reference. The ionophore and guest salt were initially at the concentrations of 3.0×10^{-2} and 1.5×10^{-2} M. Other conditions were similar to those described above. After the mixture was stirred, the $CDCl_3$ phase was separated and measured spectroscopically.

Acknowledgment. This research was supported in part by a Grant-In-Aid from the Ministry of Education, Science, and Culture of Japan.

(23) ^{13}C-NMR spectral changes also supported a 1:1 complexation with monovalent cations and a 1:2 complexation with divalent cations.

(24) Tsukube, H. *Liquid Membranes: Chemical Applications*; CRC Press: Florida, 1990; p 27.